CLAIMS

1. A multilayer enzyme immobilization process comprising:

adsorbing a polyethyleneimine solution onto a fibrous matrix, said matrix including a plurality of fibrils;

adding an enzyme in solution to said fibrous matrix;

forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils; and

cross-linking said polyethyleneimine-enzyme aggregates.

2. The process of claim 1 wherein said enzyme is selected from β-galactosidase, lipase, lactate dehydrogenase, formate dehydrogenase, glucose isomerase, and combinations thereof.

3. The process of claim 2 wherein said β-galactosidase is isolated from a microorganism selected from *B. circulans, B. singularis, T. aquaticus, K. lactis, E. coli, A. oryzae, A. niger,* or combinations thereof.

4. The process of claim 3 wherein said β-galactosidase is isolated from the microorganism *A. oryzae*, *B. circulans*, *K. lactis*, or combinations thereof.

5. The process of claim 1 wherein said fibrous matrix is selected from cotton fibers, poly(ethylene terephthalate), glass fiber, wool, carbon fiber, ceramic fiber, paper, rayon, or combinations thereof.

6. The process of claim 1 wherein said fibrous matrix comprises an enzyme load that is less than about 500 mg/g fibrous matrix after adding said enzyme solution.

7. The process of claim 1 wherein said polyethyleneimine solution comprises polyethyleneimine solubilized in water.

8. The process of claim 7 wherein the concentration of polyethyleneimine in said polyethyleneimine solution is between about 0.001 mg/mL and the solubility of polyethyleneimine in water.

9. The process of claim 7 wherein the concentration of polyethyleneimine in said polyethyleneimine solution is between about 0.001 and about 30 mg/mL.

10. The process of claim 7 wherein the concentration of polyethyleneimine in said polyethyleneimine solution is about 2 mg/mL.

11. The process of claim 1 wherein said enzyme solution comprises enzyme solubilized in water.

12. The process of claim 11 wherein the concentration of enzyme in said enzyme solution is between about 0.001 and about 100 mg/mL.

13. The process of claim 1 wherein said polyethyleneimine molecules are positively charged, said enzyme is negatively charged, and said fibrous matrix comprises a balanced charge ratio of said polyethyleneimine to said enzyme.

14. The process of claim 1 wherein said fibrous matrix comprises a ratio of polyethyleneimine to enzyme of between about 1/33.3 and about 1/8.3 after adsorbing said polyethyleneimine solution in said fibrous matrix and adding said enzyme solution.

15. The process of claim 14 wherein said fibrous matrix comprises a polyethyleneimine to enzyme ratio of between about 1/20 and about 1/25 after adsorbing said polyethyleneimine solution in said fibrous matrix and adding said enzyme solution.

16. The process of claim 15 wherein said fibrous matrix comprises a polyethyleneimine to enzyme ratio of between about 1/22 and about 1/25.

17. The process of claim 1 further comprising maintaining said polyethyleneimine and enzme solution at a pH in the range of between about 4 and about 10 during said forming at least two layers of polyethyleneimine-enzyme aggregates.

18. The process of claim 17 wherein said pH is between about 6 and about 8.

19. The process of claim 1 further comprising maintaining said enzyme solution at a temperature that is less than about 65°C prior to adding to said fibrous matrix.

20. The process of claim 19 further comprising maintaining said enzyme solution at a temperature that is between about 0 and about 25°C prior to adding to said fibrous matrix.

21. The process of claim 1 further comprising forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils at a temperature that is less than about 65°C.

22. The process of claim 1 further comprising forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils at a temperature that is between about 0°C and room temperature.

23. The process of claim 1 further comprising forming at least two layers of polyethyleneimine-enzyme aggregates on said fibrils such that said aggregates completely cover said fibrils.

24. The process of claim 1 wherein said cross-linking of said polyethyleneimine-enzyme aggregates is performed by applying an enzyme fixative.

25. The process of claim 24 wherein said enzyme fixative is an aldehyde or keto compound that can form covalent bonds with the amine groups of an enzyme protein.

26. The process of claim 24 wherein said enzyme fixative is gluteraldehyde, formaldehyde, or combinations thereof.

27. The process of claim 26 wherein said gluteraldehyde comprises a solution of gluteraldehyde and water.

28. The process of claim 27 wherein said gluteraldehyde solution has a concentration between about 0.05 and about 0.2%.

29. The process of claim 28 wherein said gluteraldehyde solution has a concentration of about 0.1%.

30. The process of claim 24 wherein said enzyme fixative has a pH between about 6 and about 8.

31. The process of claim 24 wherein said enzyme fixative is reacted with said polyethyleneimine-enzyme aggregates for a time sufficient to cross-link said polyethyleneimine-enzyme aggregates.

32. The process of claim 31 wherein said enzyme fixative is reacted with said polyethyleneimine-enzyme aggregates for at least about 5 minutes.

33. The process of claim 24 further comprising maintaining said enzyme fixative at a temperature that is less than about 65°C prior to cross-linking said polyethyleneimine-enzyme aggregates.

34. The process of claim 1 further comprising washing said fibrils and said cross-linked polyethyleneimine-enzyme aggregates formed thereon with distilled water and acidic buffer subsequent to said cross-linking.

35. The process of claim 1 wherein said process produces an immobilized enzyme yield that is less than or equal to about 100%

36. The process of claim 1 wherein said process produces an immobilized enzyme yield that is at least about 5%.

37. A process of using multilayer immobilized enzyme in the production of galacto-oligosaccharides from lactose, said multilayer immobilized enzyme being prepared by the process of claim 1.

38. A process of using multilayer immobilized enzyme in the hydrolysis of lactose to glucose and galactose, said multilayer immobilized enzyme being prepared by the process of claim 1.

39. A fibrous-bed biocatalytic reactor for production of galacto-oligosaccharides from lactose, said reactor comprising:

a fibrous matrix disposed in a vessel, wherein

said fibrous matrix comprises said multilayer immobilized polyethyleneimine-enzyme aggregates prepared by the process of claim 1, and

said vessel is configured for the steady flow of a lactose solution.

40. A fibrous-bed biocatalytic reactor for the hydrolysis of lactose to glucose and galactose, said reactor comprising:

a fibrous matrix disposed in a vessel, wherein

said fibrous matrix comprises said multilayer immobilized polyethyleneimine-enzyme aggregates prepared by the process of claim 1, and

said vessel is configured for the steady flow of a lactose solution.